Test Method 1104
Detection of Escherichia Coli in Drinking Water by the EC Medium with Mug Tube Procedure

1. Scope and Application

1.1 This method describes a procedure for the detection and enumeration of Escherichia coli (E. coli) in drinking water by transfer from total coliform-positive presumptive tests to EC + MUG medium. Because this species is a natural inhabitant only of the intestinal tract of warm-blooded animals, its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens.

1.2 This method can be applied to positive presumptive results from the multiple tube fermentation (MTF), presence-absence (P-A), and membrane filter (MF) procedures for total coliforms.

1.3 The detection limit of this procedure is one micro–organism per 100 mL.

1.4 The Total Coliform Rule[1] requires that all total coliform-positive cultures be tested for either fecal coliforms or E. coli. This method for E. coli is approved in the National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria[2].

2. Summary

2.1 Culture from total coliform-positive tubes or bottles of lauryl tryptose, lactose or P-A medium, or from total coli form MF colonies or entire surface growth in the presumptive phase of these procedures for total coliforms is inoculated into EC broth containing 4-methylumbelliferyl-ß-D-glucuronide (EC + MUG) and incubated at 44.5 ± 0.2°C for 24 hours[3]. Observance of bright blue fluorescence when subjected to long-wave (366 nm) ultraviolet (UV) light indicates a positive test for E. coli.

3. Definition

3.1 In this method E. coli are defined as those bacteria which produce bright blue fluorescence in EC + MUG medium after initial culture in lauryl tryptose, lactose or P-A broth or on Endo MF plates.

4. Interferences

4.1 Certain brands of test tubes fluoresce under long-wave UV light and may interfere with test results. Tubes should be examined before use.

4.2 Do not use an inverted vial; gas production is not relevant to the test and observation for this reaction may cause confusion in test interpretation.

5. Safety Precautions

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents and materials and while operating sterilization equipment.

6. Apparatus and Equipment

6.1 Water bath, gable-covered incubator that maintains a 44.5 ± 0.2°C temperature.

6.2 Lamp, ultraviolet, long-wave, 366 nm, preferably with a 6-watt bulb.

6.3 Inoculation loop, 3 mm diameter or needle of nichrome wire, 26 B&S gauge, in suitable holder. Sterile applicator sticks are a suitable alternative to inoculation loops or needles. Sterile cotton-tipped applicator sticks are used for the transfer of growth from the entire MF surface by the swab technique.

6.4 Bunsen or Fisher-type burner or electric incinerator unit.

6.5 Thermometer, glass/mercury or dial calibrated in 0.2°C increments or less, checked against a National Bureau of Standards (NBS) certified thermometer, or one traceable to an NBS thermometer.

6.6 Ethanol, methanol or isopropanol in small, wide-mouth container, for flame-sterilizing forceps.

6.7 Pyrex test tubes, 150 x 20 mm.

6.8 Culture tube racks to hold 20 mm diameter tubes.

6.9 Flasks, borosilicate glass, screw-cap, 250 - 1000 mL volume.

7. Media and Reagents

7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. The agar used in preparation of culture media must be of microbiological grade.

7.2 Whenever possible, use commercial culture media as a means of quality control.

7.3 EC Medium with MUG (Difco 0022-17), EC Broth with MUG (BBL 12332) or equivalent.

Composition:

Tryptose or Pancreatic Digest of Casein Peptone	20.0 g
Lactose	5.0 g
Bile Salts No. 3 or Bile Salts Mixture	1.5 g
Dipotassium Phosphate	4.0 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5.0 g
4-methyl-ß-D-umbelliferyl glucuronide (MUG)	0.05 g

Reagent Grade Water 1 L
Final pH: 6.9 ± 0.2

Preparation: Add 37.1 grams (Difco) or 37.0 grams (BBL) of EC with MUG medium or equivalent to 1 liter of water and warm slightly to dissolve completely. Dispense into tubes (150 x 20 mm). Sterilize for 12-15 minutes at 121°C (15 lbs. pressure).
Alternatively, 0.05g MUG per liter may be added to EC Medium (Difco 0314.02), EC Broth (BBL 11187) or equivalent before autoclaving.

7.4 Check test tubes before use with a 366 nm UV light to ensure they do not fluoresce.

7.5 For quality control of the medium, include a MUG-positive (E. coli) and MUG-negative (e.g., uninoculated) control for each analysis or set of analyses. Check medium before use with a 366 nm UV light to ensure it does not fluoresce.

7.6 Incubate positive and negative control cultures at 35 ± 0.5°C for 24 hours in lauryl tryptose broth. Transfer a loopful to EC medium with MUG and incubate at 44.5 ± 0.2°C for 24 hours. Read and record results.

7.7 Use prepared medium in tubes with loose-fitting closures within one week. Store sterile refrigerated medium for up to three months in screw-cap tubes/containers, and incubate stored medium overnight at 35°C before use; discard tubes with growth.

8. Sample Collection, Preservation and Holding Time

8.1 This test method is a transfer procedure from a preceeding sample analysis; therefore, it does not involve direct analysis of the water sample. Consequently, sample collection, preservation and holding time are not procedures specifically applicable to this method. However, adherence to sample collection and preservation procedures and holding time limits for the original water sample is critical to the production of valid data.

9. Calibration and Standardization

9.1 Check temperatures in incubators daily to ensure operation within stated limits.

9.2 Check thermometers at least annually against an NBS certified thermometer or one traceable to NBS. Check mercury columns for breaks.

10. Quality Control

10.1 Verify at least 5% of both MUG-positive results and turbid total coliform-positive, MUG-negative results. Verification of a pure culture may be performed by the use of API 20 E or an equivalent bacterial identification system; standard biochemical tests (e.g. citrate, indole and urease tests); serotyping after biochemical identification if desired; or the indole test at 44 °C and growth in citrate.

10.2 See recommendations on quality control for microbiological analyses in Standard Methods for the Examination of Water and Wastewater.[4]

11. Procedure

11.1 Gently swirl the presumptive total coliform tube or bottle. Using a sterile inoculating loop or wooden applicator, transfer inocula from total coliform-positive presumptive phase tubes or bottles at 24 hours (or 48 hours if needed) to EC + MUG tubes.
Transfer inocula from total coliform MF colonies with a sterile needle or wooden applicator stick. Alternatively, use a sterile cotton-tipped swab to transfer the entire surface growth from a total coliform-positive MF plate to EC + MUG tubes. Do not leave the cotton swab in the medium. Gently swirl inoculated EC + MUG tubes to ensure mixing of inoculum with medium.

11.2 Incubate inoculated EC + MUG tubes at 44.5 ± 0.2°C for 24 ± 2 hours. Tubes must be placed in the incubator within 30 minutes after inoculation. The water depth in the water bath incubator must come to the top level of the culture medium in the tube.

11.3 Detect fluorescence using an ultraviolet lamp (366 nm), preferably with a 6-watt bulb. Ensure that weak autofluorescence of medium, if present, is not misinterpreted as positive for E. coli. A MUG-positive (E. coli) and MUG-negative (e.g., uninoculated) control are necessary for each analysis. The observation of bright blue fluorescence in the EC + MUG tubes after 24 ± 2 hours constitutes a positive test for E. coli.

12. Reporting

12.1 Report the presence or absence of E. coli.

References

1. Drinking Water; National Primary Drinking Water Regulations; Total Coliforms (Including Fecal Coliforms and E. Coli); Final Rule. 40 Code of Federal Regulations (CFR) Parts 141 and 142. Federal Register 54: p. 27544, June 29, 1989.
2. National Primary Drinking Water Regulations; Analytical Techniques; Coliform Bacteria. 40 Code of Federal Regulations (CFR), Part 141, Federal Register 56, p. 636, January 8, 1991.
3. Rippey, S. R., L. A. Chandler and W. D. Watkins. 1987. Fluorometric Method for Enumeration of Escherichia coli in Molluscan Shellfish. J. Food Protection 50:685-690.
4. American Public Health Association. 1985. Standard Methods for the Examination of Water and Wastewater, 16th edition. American Public Health Association, Washington, D.C.